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AUTHOR(S):

Miyazaki, Yosuke; Numata, Hideharu

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Exhibition of circannual rhythm under constant light in the varied carpet beetle *Anthrenus verbasci*

Yosuke Miyazaki and Hideharu Numata

Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka 558–8585, Japan

Yosuke Miyazaki, Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka 558–8585, Japan

Email: ymiyazaki@hcn.zaq.ne.jp

Tel.: +81-6-6605-2573

Fax: +81-6-6605-2522

Hideharu Numata (corresponding author), Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606–8502, Japan

Email: numata@ethol.zool.kyoto-u.ac.jp

Tel.: +81-75-753-4073

Fax: +81-75-753-4113

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The varied carpet beetle *Anthrenus verbasci* shows a clear circannual pupation rhythm under light/dark (LD) 12:12. We examined whether this rhythm is exhibited under constant light (LL) of 0.002 Wm⁻² and 0.9 Wm⁻² intensities. Rhythmic pupation was not observed when the larvae were continuously maintained under LL. Moreover, the circannual rhythmicity of pupation was not observed under LL after preexposure to LD 12:12 for 2 or 4 weeks but was observed after exposure for 8 weeks. Under LL of both light intensities, as the preexposure to LD 12:12 was longer, the first pupation peaks occurred earlier and almost synchronized by preexposure for 8 weeks with the first peak under continuous LD 12:12. However, the transition from LD 12:12 to LL did not reset the phase of this circannual rhythm. Preexposure to LD 12:12 probably synchronizes the asynchronous rhythm observed under LL so that a clear circannual pupation rhythm is exhibited.

Keywords: *Anthrenus verbasci*; biological clock; circannual rhythm; constant light; photoperiod

Introduction

Circannual rhythms, which control biological seasonality, occur with an endogenous period of about a year, under constant conditions without seasonal changes. To entrain a circannual rhythm to the natural year, the circannual oscillator is reset by a seasonal zeitgeber. In many species, the change of photoperiods is the most prominent zeitgeber (Gwinner 1986; Goldman et al. 2004; Paul et al. 2008). Circannual-rhythm studies have mostly been conducted under constant photoperiods, e.g. 12 h light and 12 h darkness (LD 12:12). However, circannual rhythms in some vertebrates evidently persist even under constant light (LL) (Goss 1969; Ducker et al. 1973; Pengelley et al. 1976; Chandola et al. 1982; Davis and Swade 1983; Holberton and Able 1992; see Gwinner 1986). Circannual rhythmicity observed under LL indicates that daily light-dark cycles are not necessary to sustain an endogenous rhythm.

The properties of circannual rhythm in insects have been studied by the pupation of the varied carpet beetle *Anthrenus verbasci* (L.) (Coleoptera, Dermestidae) (e.g. Blake 1959; Nisimura and Numata 2001; Miyazaki et al. 2007). Nisimura and Numata (2001) continuously maintained larvae under constant dim light (dim LL) of 0.006 Wm^{-2} intensity and found that pupation was less synchronous, and the periodic pattern was less clear than when the larvae were continuously maintained under LD 12:12. They suggested that this unclear rhythm could result from damping out of the circannual rhythm, as observed in the case of circadian eclosion rhythm under LL in flies, e.g. *Drosophila pseudoobscura* and *Sarcophaga argyrostoma* (Pittendrigh 1966; Saunders 1976). However, there is another possibility; because LL, unlike photoperiods such as LD 12:12, is not a suitable seasonal cue, in larvae maintained continuously under LL, the proper phase of the circannual oscillator cannot be set, resulting in variable pupation times among individual larvae. To

investigate the latter possibility, it is necessary to observe the circannual pupation rhythm of *A. verbasci* under LL after individual oscillators are synchronized by the photoperiod.

Recently, Miyazaki et al. (2009) showed that exposure to LD 12:12 for 8–9 weeks is sufficient to enhance the synchronous pupation of *A. verbasci* larvae. We assume that preexposure to LD 12:12 synchronizes this circannual rhythm, and that the rhythm persists during subsequent exposure to LL. Therefore, in the present study, we exposed hatching larvae to LD 12:12 for various periods and then transferred them to dim LL of 0.002 Wm^{-2} intensity or constant bright light (bright LL) of 0.9 Wm^{-2} intensity.

Materials and methods

Adults of *A. verbasci* were collected in Osaka City, Japan (34.6°N , 135.5°E) in April and May 2002–2005 and their progeny larvae were used for the experiments. The newly emerged adults and their eggs were maintained under LD 16:8 at $25 \pm 1^{\circ}\text{C}$ and within a week after hatching the larvae were transferred to experimental conditions at $20 \pm 1^{\circ}\text{C}$. The photoperiod was generated by white fluorescent lamps and timers. The light intensity in the photophase was 0.9 Wm^{-2} . To generate dim light of 0.002 Wm^{-2} intensity, the fluorescent lamp was covered with black polyethylene sheets. The relative humidity was maintained at about 66% with a saturated solution of NaNO_2 . Dried bonito powder and dried yeast were used as food for the larvae. The pupation of larvae was recorded each week (Nisimura and Numata 2001).

The differences in larval duration were examined statistically by the Steel–Dwass test for nonparametric multiple comparison with a significance level of $P = 0.05$ (Hochberg and Tamhane 1987).

Results

Transfer to constant dim light

Larvae maintained continuously under LD 12:12 pupated synchronously, with clear circannual periodicity. Larvae that pupated 23–27 weeks after hatching were classified under the first pupation group, and the median larval duration of this group was 25 weeks. Larvae of the second group pupated 54–68 weeks after hatching, and their median larval duration was 62 weeks. The interval between the medians of the two pupation groups was 37 weeks (Figure 1f). However, when the larvae were maintained continuously under dim LL, only one pupation group was observed 27–44 weeks after hatching; the median larval duration of this group was 31 weeks, which was significantly more than that of the first group under continuous LD 12:12 (Figure 1a). When larvae were transferred from LD 12:12 to dim LL, 2 and 4 weeks after hatching, pupation occurred 26–49 weeks after hatching except in the case of one individual, and the median larval duration was 33 and 28 weeks, respectively (Figure 1b, c). Larvae transferred to dim LL, 6 and 8 weeks after hatching, mostly pupated 23–33 weeks after hatching and were classified under the first pupation group in each condition, and their median larval duration was 26 and 25 weeks, respectively. The pupation times were significantly less than the pupation time under continuous dim LL but not significantly different from that of the first group under continuous LD 12:12. Thus, preexposure to LD 12:12 reduced the difference between the pupation time under dim LL and that of the first group under continuous LD 12:12. Several remaining larvae pupated 70–83 weeks after hatching and were classified as the second pupation group in each condition. When larvae were transferred to dim LL, 6 and 8 weeks after hatching, the median larval duration of the second group was 74

and 77 weeks, and the intervals between the first and the second groups were 48 and 52 weeks, respectively (Figure 1d, e). Thus, clear rhythmic pupation was observed under dim LL after exposure to LD 12:12 for 6 or 8 weeks.

Transfer to constant bright light

Under continuous LD 12:12 exposure, the median larval durations of the first and the second pupation groups were 24 and 60.5 weeks, respectively, and the interval was 36.5 weeks (Figure 2h). When larvae were continuously maintained under bright LL without preexposure to LD 12:12, only one pupation group was observed. Most larvae pupated 27–48 weeks after hatching, and the median larval duration was 35 weeks; this duration was significantly longer than that of the first group under continuous LD 12:12. The pupation was less synchronous than that under continuous LD 12:12 (Figure 2a). When larvae were transferred from LD 12:12 to bright LL, 2 weeks after hatching, pupation occurred 4 weeks later than that under continuous bright LL (Figure 2b). When larvae were transferred to bright LL, 4 or 6 weeks after hatching, bimodal distribution of pupation was observed, spanning 24–74 weeks after hatching. Therefore, preexposure to LD 12:12 for 4 or 6 weeks after hatching split the distribution of pupation under bright LL even though the distribution was not distant enough to establish circannual periodicity (Figure 2c, d). When larvae were transferred to bright LL, 8, 10, and 12 weeks after hatching, pupation showed a clear circannual rhythm with intervals of 34.5, 35, and 41 weeks between the median larval durations of the two pupation groups, respectively. The first group comprised larvae that pupated 18–36 weeks after hatching, and the median larval duration was 26 weeks. Most of the remaining larvae pupated 49–86 weeks after hatching and were classified as the second group. Among these three conditions, there was no significant

difference between the median larval duration of the first and the second groups (Figure 2e–g). Thus, preexposure to LD 12:12 for 8 weeks was necessary for clear circannual pupation rhythm under bright LL. The longer the duration of LD 12:12 preexposure, the shorter was the time required for first pupation to occur. The pupation of the first group occurred significantly later and was less synchronous than that of the first group under continuous LD 12:12 (Figure 2e–h); this result was different for larvae transferred to dim LL (Figure 1d–f).

Discussion

Common features of the circannual pupation rhythm of *A. verbasci* and the circadian eclosion rhythm of *Drosophila pseudoobscura*, e.g. self-sustaining oscillation, temperature compensation, and phase-dependent phase shifts according to the zeitgeber, have been reported (Nisimura and Numata 2001; Miyazaki et al. 2005, 2007). Although the eclosion rhythm of *D. pseudoobscura* is rapidly damped out under LL (Pittendrigh 1966), the periodicity can be clearly observed over four or five cycles, upon transfer of pupae from constant darkness (DD) to LL just before eclosion (Engelmann 1966; Chandrashekar 1967; Chandrashekar and Loher 1969). In the present study, rhythm in the pupation of *A. verbasci* was not observed under continuous LL, whereas it was clearly observed under LL after preexposure to LD 12:12 for 8 weeks. Therefore, the circannual oscillator of *A. verbasci* can be driven over two cycles under LL.

The circannual pupation rhythm of *A. verbasci* was less synchronous at the higher light intensity than at the lower one. Moreover, while an 8-week exposure to LD 12:12 was sufficient to synchronize the first pupation peak under dim LL with that under continuous LD 12:12 (Figure 1e, f), even a 12-week exposure was not sufficient

to synchronize the first pupation peak under bright LL with that under continuous LD 12:12 (Figure 2g, h). Therefore, a higher intensity is more effective in disrupting the circannual rhythm under LL. In the circadian eclosion rhythm of *D. pseudoobscura*, the eclosion peaks are broader when the light intensity of constant dim blue light is higher (Winfree 1974). In the present study, light intensity also affected the circannual period of *A. verbasci*. After LD 12:12 preexposure for 8 weeks, the endogenous period under bright LL (34.5 weeks) was considerably shorter than that under dim LL (52 weeks). In some species, the endogenous period of the circadian rhythm has been observed to depend on light intensity (Saunders 2002; Johnson et al. 2003), including the eclosion rhythm of *D. pseudoobscura* (Chandrashekar and Loher 1969). However, in the circannual rhythms of the ground squirrels *Spermophilus lateralis* and *S. beecheyi*, the endogenous period is not affected by light intensity under LL (Pengelley et al. 1976; Davis and Swade 1983). Therefore, the circannual oscillator of *A. verbasci* seems more sensitive to light intensity than that of ground squirrels. However, it is unclear whether the disruption of rhythm at the higher light intensity is directly linked to the endogenous period.

We also report a new finding about the effect of transfer to LL on the circannual rhythm of *A. verbasci*. In previous studies, a significant phase shift in the circannual rhythm was induced upon transfer from LD 12:12 to LD 16:8, 8–10 weeks after hatching (Nisimura and Numata 2001; Miyazaki et al. 2005, 2007, 2009). However, we observed that when larvae were transferred from LD 12:12 to bright LL, 8–10 weeks after hatching, the pupation time was only slightly delayed in comparison to the pupation time under continuous LD 12:12. Therefore, it is unlikely that transfer from LD 12:12 to LL acts as an effective zeitgeber to reset the phase in *A. verbasci*. Similar results were obtained upon transfer to DD (Miyazaki et al. 2009). Thus, the

circannual phase of *A. verbasci* is only weakly sensitive to LL and DD. This is different from the circannual rhythms of the European starling *Sturnus vulgaris* and the rainbow trout *Oncorhynchus mykiss* (Gwinner 1973; Randall et al. 1998). In *S. vulgaris*, exposure to LL for 1 month under natural daylength and temperature affects testicular development in a phase-dependent manner, and the effect of LL on testicular development is different from that of DD (Gwinner 1973). *O. mykiss* interprets LL as long days and shows phase-dependent phase shifts in the spawning rhythm upon exposure to LL for 2 months under natural daylength and temperature (Randall et al. 1998; see also Duston and Bromage 1986). Unlike these two species, *A. verbasci* probably does not perceive LL and DD as suitable seasonal cues but perceives complete photoperiods, such as LD 12:12 and LD 16:8, as an appropriate zeitgeber for entrainment to the natural annual cycle.

Nisimura and Numata (2001) observed a weak circannual pupation rhythm in *A. verbasci* under dim LL of 0.006 W m^{-2} intensity. Although the present results did not show a circannual pupation rhythm under continuous LL, it is possible that a circannual oscillator does not stop and is driving in a partially asynchronous state among individual larvae. Under bright LL, preexposure to LD 12:12 for 4 or 6 weeks resulted in bimodal distribution of pupation. This suggests that phase-dependent phase shifts in asynchronous oscillating rhythms are caused by exposure to LD 12:12 (Miyazaki et al. 2006). We previously reported that a photoperiodic stimulus similarly splits less synchronous population into advanced and delayed groups (Miyazaki et al. 2005). Therefore, under bright LL, preexposure to LD 12:12 for 4 or 6 weeks is sufficient to cause phase-dependent phase shifts of an asynchronous population but not sufficient to synchronize the rhythm completely. However, preexposure to LD 12:12 for 8 weeks resets the circannual rhythm of an asynchronous population to the

same phase so that a clear circannual rhythm is exhibited. In summary, the circannual rhythm of *A. verbasci* oscillates under LL, although the initial circannual phase of newly hatched larvae is partially different among individuals. Therefore, for the circannual rhythm to be exhibited clearly under LL, it is necessary to reset individual rhythms to a particular phase by preexposure to photoperiodic seasonal cues, such as LD 12:12.

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Figure 1. Pupation of *Anthrenus verbasci* under dim LL of 0.002 Wm^{-2} intensity. Larvae were transferred from LD 12:12 to dim LL at various time points between 0 and 8 weeks after hatching (a–e) or maintained continuously under LD 12:12 (f) at 20°C . Each filled bar indicates the duration of LD 12:12 exposure. The numerals above the vertical lines indicate the number of larvae remaining. The triangle indicates the median larval duration of each group. The vertical dotted lines pass through the medians of the groups of larvae maintained continuously under LD 12:12. The median values in the group indicated with the same letter are not significantly different ($P > 0.05$; Steel–Dwass test).

Figure 2. Pupation of *Anthrenus verbasci* under bright LL of 0.9 Wm^{-2} intensity. Larvae were transferred from LD 12:12 to bright LL at various time points between 0 and 12 weeks after hatching (a–g) or maintained continuously under LD 12:12 (h) at 20°C . Each filled bar indicates the duration of LD 12:12 exposure. The numerals above the vertical lines indicate the number of larvae remaining. The triangle indicates the median larval duration of each group. The vertical dotted lines pass through the medians of the groups of larvae maintained continuously under LD 12:12. The median values in the group indicated with the same letter are not significantly different ($P > 0.05$; Steel–Dwass test).

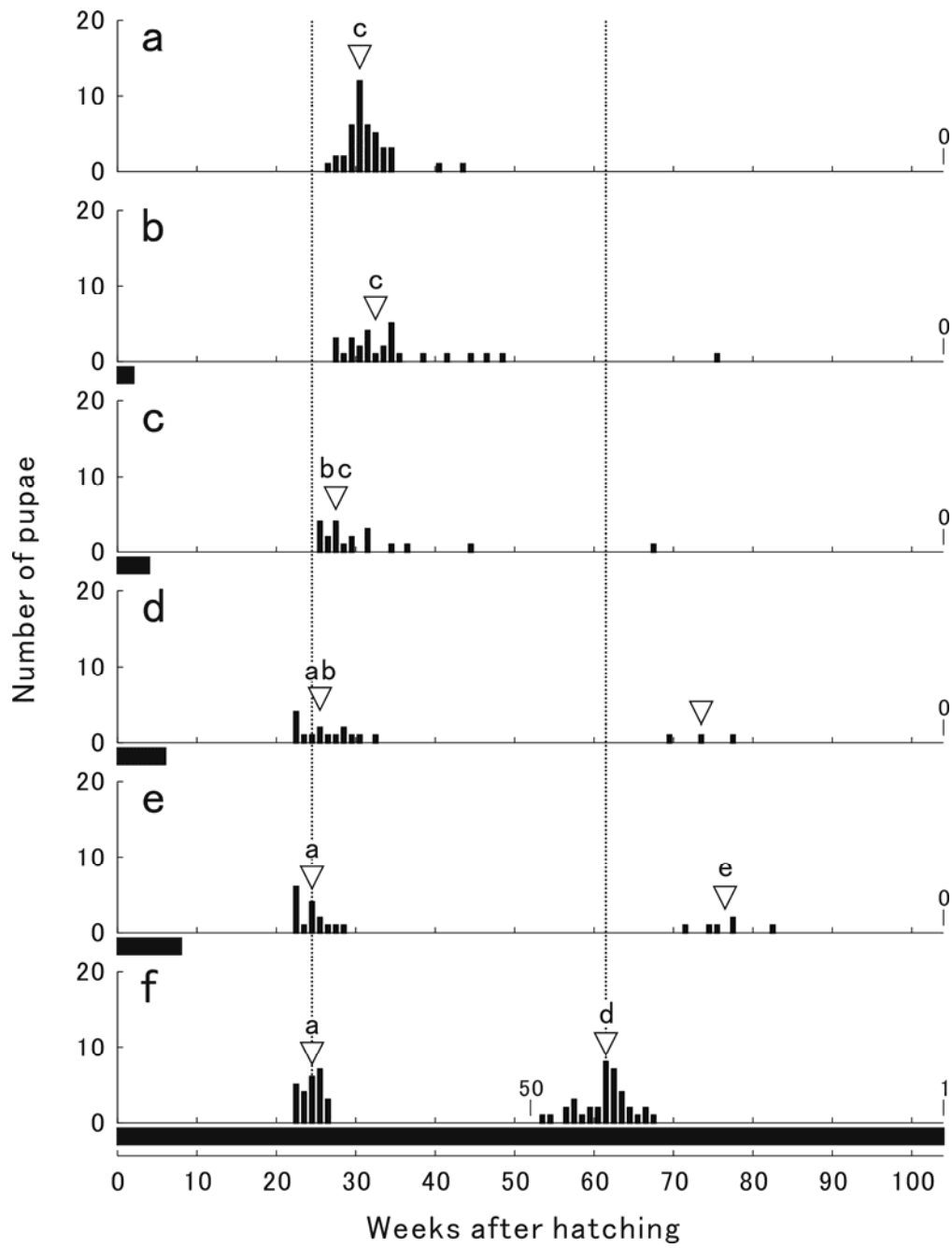


Figure 1

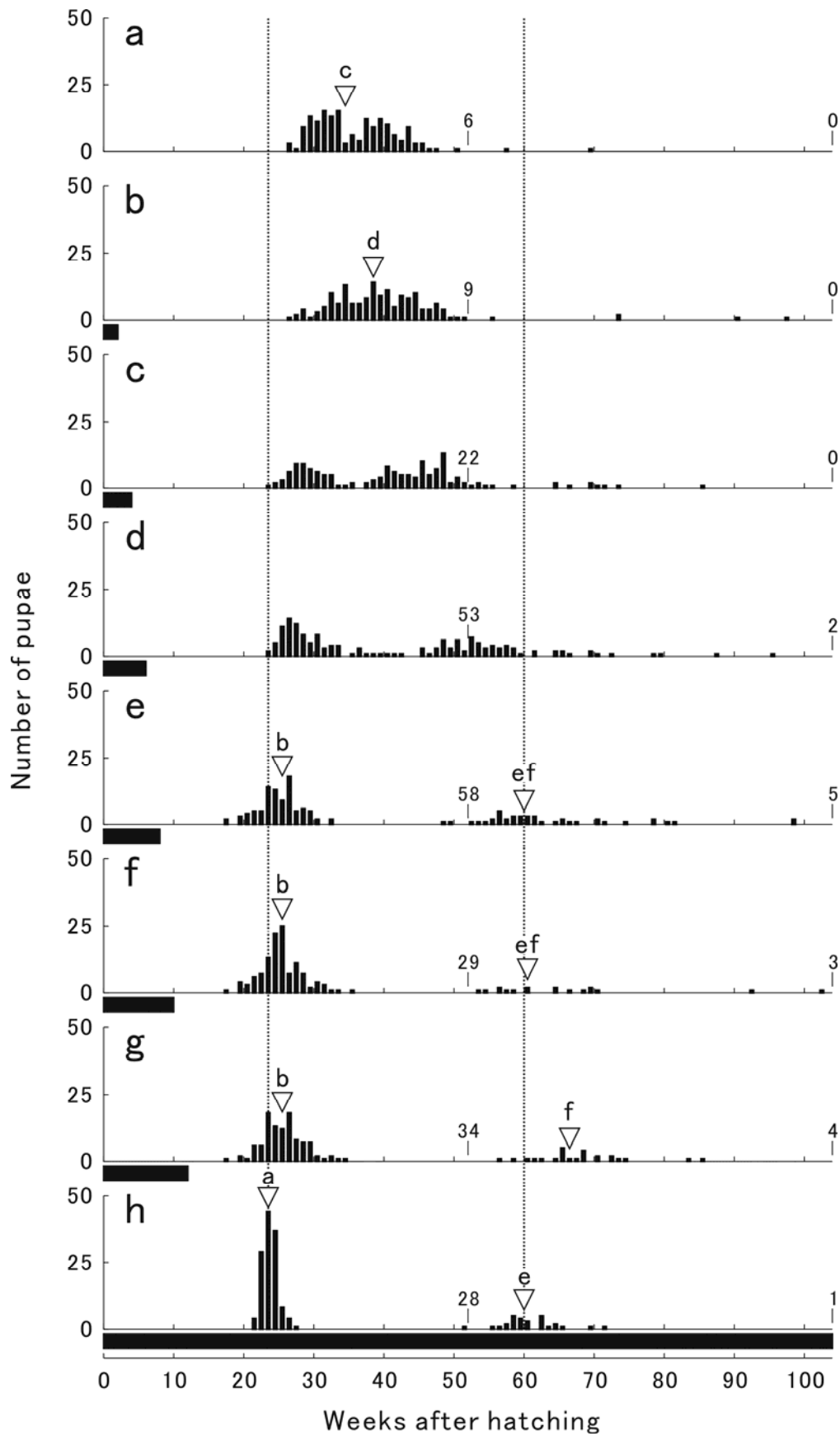


Figure 2